KODA20A.001AUS PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Tanimoto, et al.	Group Art Unit 1651
Appl. No.	:	09/514,999	
Filed	:	February 29, 2000	
For	:	METHOD OF MANUFACTURING POLYAMINE COMPOSITION	
Examiner	:	I. Marx	

DECLARATION UNDER 1.132

Assistant Commissioner for Patents

Washington, D.C. 20231

Dear Sir:

I, Yoshihiro Tanimoto, a co-inventor of the above-identified application, do hereby state and declare as follows:

1 The following comparative example and example of the claimed invention were conducted by me or under my direct control or supervision:

2 The difference between the comparative example and the example resided solely in the absence or presence of a decomposition step by nuclease digestion. The example shows that the yield of polyamines was 4.2 times as compared with the comparative example.

3 Comparative Example: Polyamines were obtained from yeast ribonucleic acid (Kirin Beer K.K.) as yeast somatic components as follows:

4 The yeast ribonucleic acid was dissolved in a 10 mM sodium acetate buffer solution (pH 5) to adjust the concentration of the yeast ribonucleic acid to 5%. The yeast ribonucleic acid solution obtained was then introduced into a column filled with a cation-exchange resin (Dowex 50WX8(H+Type)) to absorb polyamines onto the cation-exchange resin. Thereafter, the column was sufficiently washed with a 0.5M NaCl solution to fully remove non-absorption materials from the column, and then polyamines were eluted using 6N HCl. Sodium

hydroxide was added to neutralize the elute obtained, and then desalination was conducted by electrodialysis. After freeze-drying, 550 mg of a polyamine composition was obtained per 1 kg of yeast ribonucleic acid. The composition contained 460 mg of polyamines. In the polyamines, the total amount of spermidine and spermine was 437 mg (95%).

5 Example: Polyamines were obtained from yeast ribonucleic acid (Kirin Beer K.K.) as yeast somatic components as follows:

6 The same processes as in the comparative example were conducted except that a decomposition step by nuclease digestion was conducted before the column purification. The decomposition step was as follows: Nuclease was added to the 5% yeast ribonucleic acid solution to give a nuclease concentration of 1% by weight, and then enzymatic hydrolysis was conducted at 37°C for 72 hours, before introducing the hydrolysis solution obtained into the column.

7 After freeze-drying, 2273 mg of a polyamine composition was obtained per 1 kg of yeast ribonucleic acid. The composition contained 1932 mg of polyamines. In the polyamines, the total amount of spermidine and spermine was 1855 mg (96%). That is 4.2 times as high as the yield in the Comparative Example.

8 I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated: October 21, 2002

By: Yoshihiro Tanimoto
Yoshihiro Tanimoto

I:\DOCS\KOA\KOA-4643.DOC
102002